環境ストレス・免疫応答における視床下部・脾臓交感神経系の機能的連関

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発表論文

The 5-HT_{1A} receptor agonist, 8-OH-DPAT, attenuates stress-induced anorexia in conjunction with the suppression of hypothalamic serotonin release in rats

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Abstract

The effect of the selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) on stress-induced anorexia and serotonin (5-HT) release in the rat hypothalamus was studied with brain microdialysis. Subcutaneous injection of 8-OH-DPAT (1 mg/kg) significantly attenuated the immobilization-induced anorexia for 3 h, but had no effect during the following 9 h. Injection of 8-OH-DPAT itself had no effect on basal release of 5-HT, while it significantly blocked the immobilization-induced 5-HT release in the lateral hypothalamus. The results suggest that 8-OH-DPAT attenuated the stress-induced anorexia through the activation of 5-HT_{1A} autoreceptors in dorsal raphe nucleus.

***Theme*:** Neural basis of behaviour

***Topic*:** Ingestive behaviours

Keywords: Immobilization stress; Serotonin; 8-OH-DPAT; Food intake; Lateral hypothalamus; In vivo microdialysis

Significant suppression of food intake and body weight loss has been reported to occur after immobilization-stress in rats [16,19,25,28]. While the precise mechanisms involved in the stress-induced anorexia have not been fully elucidated, activation of the serotonin (5-hydroxytryptamine, 5-HT) pathway in the brain may contribute to the mechanism(s). A significant increase in hypothalamic 5-HT release occurred in response to the immobilization stress in rats [27,29], and chronic recording of neuronal activity demonstrated that a large proportion of the hypothalamic neurons were inhibited by acute immobilization, which was significantly antagonized by preinjection of a non-selective 5-HT receptor antagonist, methysergide [28]. This evidence supports the hypothesis that the immobilization-induced anorexia is mediated, at least in part, through an increase in the functional activity of serotonergic neurons in the hypothalamus which is known to be important in the regulation of food intake [2,21].

Converging pharmacological evidence supports an inhibitory role for 5-HT in the control of food intake [3,9,20,26,30]. Pharmacological manipulations that increased the availability of 5-HT in synaptic terminals suppressed feeding behavior, while decreased 5-HT receptor activation had the opposite effect. Among the orexigenic agents, a potent and selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), had a facilitative effect on food intake by decreasing 5-HT synthesis and release via activation of 5-HT autoreceptors [1,6,7]. Activation of the 5-HT_{1A} autoreceptors of the raphe nuclei has been shown to decrease raphe neuronal activity [11,31] and to decrease the synthesis and release of 5-HT in the forebrain [13,14]. Based on these findings, the present study was designed to examine the hypothesis that 8-OH-DPAT attenuates the

immobilization-induced anorexia in conjunction with the suppression of 5-HT release in the lateral hypothalamic area (LHA) of awake rats.

Male Wistar rats that weighed 250 to 300 g at the beginning of the experiments were used. Before the experiments, the rats were housed in individual cages with artificial illumination from 08:00 h to 20:00 h and ambient temperature at 23±1 °C. They were maintained ad libitum on powdered food (CE2; Japan Clea Inc. Ltd.) and tap water except between 17:30 h and 20:00 h when food and water were removed. Premeasured food and water were presented at 20:00 h. Food intake was measured at 23:00 h (3-h intake) and at 08:00 h (9-h intake). The rats were immobilized by strapping their paws to restraining boards with adhesive tape, as described previously [24]. Immobilization was started at 17:30 h and continued for 2 h.

One week before the experiments, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a guide cannula was stereotaxically implanted for penetration of a microdialysis probe into the LHA. The coordinates were 4.6±0.3 mm anterior from the interaural line, 1.5±0.5 mm lateral from the midsagittal sinus, and 6.0 mm ventral from the cortical surface according to the atlas of König and Klippel [18]. After positioning the tip of the guide cannula just above the LHA, it was fixed to the surface of the skull with dental acrylic cement. Rats were allowed to recover for one week before the microdialysis experiments. Hollow fibers (acetate cellulose; mol. wt. cut off , 50 kDa) with 220 μm o.d. were used to prepare the microdialysis probe. The evening before the experiment, the rats were lightly anesthetized with ether and a microdialysis probe was inserted through the guide shaft and fixed in place. The tip of the probe extended 3 mm beyond the guide shaft to reach the LHA. A microinjection pump, with a gas tight

syringe, perfused Ringer solution at a rate of 2 μl/min. The histological examination after the experiment verified the correct location of the dialysis membrane. Dialysis samples were analyzed by reversed-phase high-performance liquid chromatography (HPLC) with electrochemical detection (Eicom, Kyoto, Japan). 8-OH-DPAT, dissolved in physiological saline, was injected subcutaneously (s.c.) in volume of 0.1 ml/100 g body weight. For the feeding behavior experiments, doses of 8-OH-DPAT were divided into halves which were injected one hour before (16:30 h) and just after the immobilization (19:30 h). Controls received an equivalent volume of saline.

All data are given as the mean±S.E.M. Statistical analysis was performed using a one-way analysis of variance (ANOVA) with repeated measures and Dunnett's multiple range test for *post-hoc* determination of significant differences. A two-way ANOVA with repeated measures was also used when appropriate, and comparisons of individual groups at corresponding concentrations or times in different groups were carried out with a one-way ANOVA and Bonferroni/Dunn multiple range test for *post-hoc* determination of significant differences. A P value of less than 0.05 indicates statistical significance.

To determine whether activation of 5-HT_{1A} receptors might modify the immobilization-induced anorexia, the effects of 8-OH-DPAT on immobilization-induced anorexia was evaluated. As shown in Fig. 1A, injection of physiological saline plus immobilization significantly decreased 3-h and the following 9-h food intake compared to the control. Food intake decreased to 57.9±4.0% (n = 8) of the control amount during the 3-h period [physiological saline, 6.8±0.4 g; physiological saline plus immobilization, 3.9±0.2 g; $F(1, 14)=45.07$, $P < 0.01$, one-way ANOVA] and to 61.7±5.5% of control for the following 9-h

period [physiological saline, 16.2±0.6 g; physiological saline plus immobilization, 10.1±1.1 g; $F(1, 14)=19.59$, $P < 0.01$]. While 8-OH-DPAT did not increase feeding in the controls it significantly antagonized immobilization-induced anorexia for 3 h [8-OH-DPAT, 6.5±0.4 g; 8-OH-DPAT plus immobilization, 6.4±0.3 g; $F(1, 14)=0.03$, $P > 0.1$]. Difference in 3-h food intake between physiological saline plus immobilization and 8-OH-DPAT plus immobilization was statistically significant [$F(1, 14)=62.92$, $P < 0.01$]. During the following 9 h, however, 8-OH-DPAT could not antagonize the immobilization-induced anorexia, and immobilization significantly reduced food intake compared to the control [8-OH-DPAT, 15.6±0.7 g; 8-OH-DPAT plus immobilization, 8.6±0.8 g, $F(1, 14)=34.12$, $P < 0.01$]. There was no significant difference between physiological saline plus immobilization and 8-OH-DPAT plus immobilization groups for 9-h food intake [10.1±1.1 g and 8.6±0.8 g, respectively; $F(1, 14)=1.01$, $P > 0.1$]. A dose-related antagonism of 8-OH-DPAT on immobilization induced anorexia was observed for the 3-h intake [$F(4, 35)=12.12$, $P<0.01$, one-way ANOVA], but no significant effect was observed for the following 9-h intake [$F(4, 35)=0.55$, $P > 0.1$] (Fig. 1B). A two-way ANOVA with repeated measures revealed a significant difference in the effect of 8-OH-DPAT on 3-h intake and 9-h intake [$F(1, 70)=44.24$, $P < 0.01$].

(Figure 1 about here)

The time course of changes in 5-HT levels in the LHA before, during and after immobilization stress are shown in Fig. 2. Changes in the concentration of 5-HT in the dialysate are expressed as a percent of the mean basal release. The basal values for 5-HT were calculated from

the mean concentrations of three consecutive dialysates before the injection of 8-OH-DPAT. The basal level of 5-HT was 1.2±0.3 pg/40 μl of perfusate (n = 10), and it was fairly stable before immobilization. In contrast, immobilization stress rapidly and significantly increased the concentration of 5-HT in the LHA. The level reached a maximum of 396.6±71.9% (n = 5) 40 min after the start of the immobilization, and began to decrease toward the pre-immobilization level even while the immobilization stress still continued. At the end of the immobilization for 120 min the increased level was 220.0±56.7% of the basal release, which gradually returned to the basal level. Dunnett's multiple range test for *post-hoc* determination of significant differences indicated that immobilization-induced 5-HT release was statistically significant for 80 min after the start of the immobilization. Further experiments were conducted to examine whether 8-OH-DPAT would attenuate the immobilization-induced 5-HT release in the LHA. Pre-injection of 8-OH-DPAT (1 mg/kg, s.c.) significantly blocked the immobilization-induced 5-HT release. A two-way ANOVA with repeated measures revealed a significant difference between the groups (physiological saline plus immobilization vs. 8-OH-DPAT plus immobilization; $F(1,136)=88.94$, $P < 0.01$). Injection of 8-OH-DPAT itself had no effect on basal release of 5-HT which was fairly stable during the 280-min experimental period.

(Figure 2 about here)

The present study confirmed that pre-injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT significantly attenuated the immobilization-induced anorexia in conjunction with the suppression

of 5-HT release in the LHA. Injection of 8-OH-DPAT did not increase feeding in the controls which might be due to diurnal variations in the feeding response to 8-OH-DPAT [5]. Further the rats were food deprived for 2.5 h just before the dark period of the L/D cycle, and it has been reported that the orexigenic effect of 8-OH-DPAT was observed only in freely feeding rats [1,6,7].

While the exact mechanisms by which 8-OH-DPAT attenuates stress-induced anorexia have not been fully elucidated, a possible site of action for 8-OH-DPAT seems to be the dorsal raphe nucleus (DRN) [7]. Converging pharmacological evidence indicated that reductions of 5-HT release induced by systemic administration of 8-OH-DPAT were mediated through the activation of somatodendritic 5-HT_{1A} autoreceptors located in the dorsal and medial raphe nuclei [4,11,12]. Injection of 8-OH-DPAT activates the 5-HT_{1A} autoreceptors which, in turn, leads to inhibition of the activity of 5-HT-containing neurons in the DRN. The DRN contains the highest density of 5-HT neurons in the brain and ascending axon of the DRN project to the forebrain, including the hypothalamus [34]. Because the lateral hypothalamus receives inhibitory 5-HT inputs from the DRN through 5-HT_1 receptors [15], local administration of 8-OH-DPAT into the DRN may cause an increase in the activity of hypothalamic neurons by disinhibition occurring at the level of LHA. The functional significance of the LHA in the regulation of feeding behavior is well established [2,21], and these neural mechanisms may explain the induction of feeding behavior following local administration of 8-OH-DPAT into the DRN.

Exposure to diverse stressors have been reported to increase 5-HT efflux in projection regions of the DRN [17,29,35], and the neural circuitry activated during various stressors has been studied extensively

by mapping the induction of Fos [22,23]. The protein Fos is the product of the immediate-early gene c-*fos*, and Fos immunohistochemistry has become a popular technique for assaying neural activity [8]. Double-labeling of neurons for 5-HT and Fos was conducted to assess activation specifically in 5-HT neurons, and the immunohistochemical detection of Fos demonstrated that uncontrollable stressors activate 5-HT-immunoreactive cells in the DRN of rats [10]. This result clearly excluded the possibility that the release of 5-HT within DRN could be independent of depolarization [33] or 5-HT released in the DRN could originate in other raphe nuclei that project to DRN [32].

In summary, the present results suggest that pre-injection of 8-OH-DPAT blocks immobilization-induced increase in 5-HT neuronal discharge in the DRN through the activation of 5-HT_{1A} autoreceptors which, in turn, results in suppression of 5-HT release from the nerve terminals in the LHA. The decrease in 5-HT release in the LHA may explain the attenuation of anorexia caused by immobilization stress. It is possible that different kinds of stress modify the metabolism of various neurotransmitters and neuropeptides in the hypothalamus. Further study will be required to clarify the mechanisms of the change in feeding behavior following acute stress.

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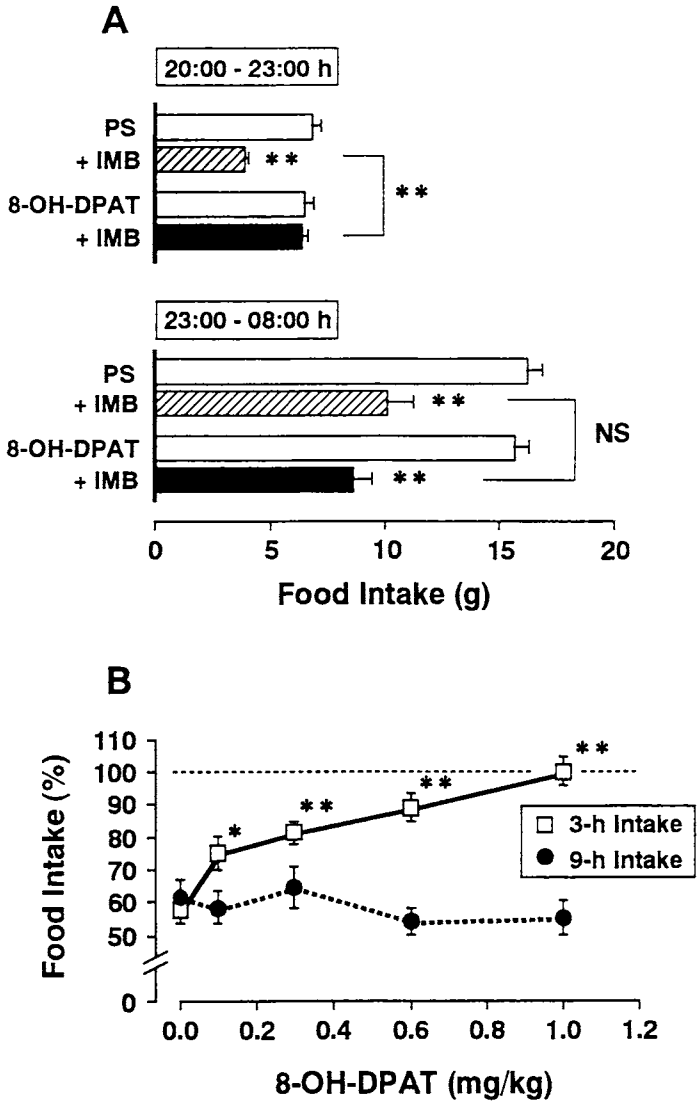

Fig. 1. Effects of 8-OH-DPAT on immobilization-induced anorexia. (A) Food intake during 3-h period (20:00-23:00 h, upper column) and the following 9-h period (23:00 - 08:00 h, lower column) after immobilization stress (n = 8 for each group). PS; physiological saline. IMB; immobilization. **, significant difference from control values ($P < 0.01$, one-way ANOVA). NS; no significant difference. (B) Dose-related antagonism of immobilization-induced anorexia after subcutaneous injection of 8-OH-DPAT. Food intake is expressed as a percentage of the control intake. *$P < 0.05$, **$P < 0.01$ vs. PS injection (one-way ANOVA followed by Dunnett's multiple-range test). Injection of 8-OH-DPAT antagonized immobilization-induced anorexia only for 3 h.

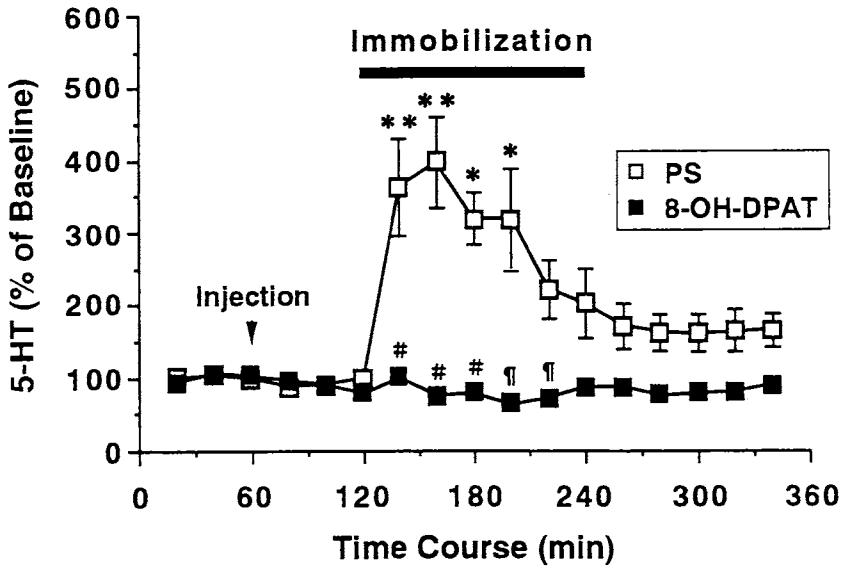

Fig. 2. Antagonism of immobilization-induced 5-HT release in the LHA by 8-OH-DPAT. Ordinate: changes in 5-HT level expressed as a percentage of the mean concentration of 3-consecutive dialysates before injection of 8-OH-DPAT. Empty squares: control, injection of physiological saline (n = 5). Filled squares: injection of 8-OH-DPAT (1 mg/kg, s.c.; n = 5). Injections were made 60 min before immobilization, indicated by arrow. Duration of immobilization is indicated by the horizontal bar. $^{**}P < 0.01$, $^{*}P < 0.05$ vs. basal release (one-way ANOVA followed by Dunnett's multiple-range test). A two-way ANOVA with repeated measures indicated a significant difference between the groups ($P < 0.01$). $\#P < 0.01$, $\P P < 0.05$; difference from the corresponding values of the control group.